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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1632	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	04/11/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	09/995,452	BENVENISTY ET AL.	
	Examiner	Art Unit	
	Thaian N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 December 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-17 and 59-64* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,11-17 and 59-64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' Response and Amendment, filed 12/27/06, has been entered. Claims 1, 11, 16, 17 are amended; claim 36 is cancelled; claims 60-64 are newly added; claims 1-9, 11-17 and 59-64 are pending and under current examination.

This action is non-final.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9, 11-17, 59-61 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is maintained for reasons of record, advance in the prior Office action, mailed

This is a new matter rejection. 37 CFR 1.118 (a) states that "No amendment shall introduce new matter into the disclosure of an application after the filing date of the application".

Applicants' Arguments. Applicants argue that the amendment to the claims does not introduce new matter into the as-filed disclosure, because the invention includes transfecting cells in any manner, such as by means of a cationic polymer transfection or by electroporation, and the specification, specifically states in ¶43 that, while electroporation is the method of choice for introducing foreign DNA into murine ES cells, the present inventors found that improved results were obtained by transfection in the presence of cationic polymers.

The amendment to the claims introduces new matter into the disclosure with regard to the recitation that the method of altering gene expression requires a transfection efficiency greater than obtainable by means of electroporation. The citations of the specification that Applicants point to fail to describe the as-amended claims such that one of skill in the art would recognize that Applicants had possession of the claimed invention. Although the specification broadly contemplates utilizing a variety of transfection reagents (see page 3, lines 11-14, for example) in order to transfect cells, and that ¶ 43 recites that utilizing cationic polymers produced improved results of transfection over electroporation, the specification only provides support to show that ExGen500 was the *only* cationic polymer that provided a greater transfection efficient than that which is obtainable by electroporation. It is reiterated that the other transfection reagents (Fugene and Lipofectamine) did not produce greater relative transfection efficiency than electroporation alone. Thus, in the context of this claimed invention as a whole, one of skill in the art would recognize that although the specification provides description only for the ExGen 500 (cationic non-lipid polymer) as a transfection reagent that fulfills the limitations of the claims. The amendment to the claims that encompasses other transfection reagents that provide transfection efficiencies greater than that obtainable by electroporation are not described nor supported by the as-filed disclosure, and thus, constitute the introduction of new matter into the presently-filed disclosure.

Applicants' Arguments. Applicants' argue that that one of ordinary skill in the art would understand that experiments with other transfection reagents could be performed in order to provide an increased efficiency over electroporation. Further, Applicants provide Darr *et al.*, post-filing art, where transfection was obtained with calcium phosphate. Applicants argue that the expression in question does not represent new matter, because it is at least implicitly present in the

specification and to those of ordinary skill in the art hat the inventors considered this to be part of their invention. See page 10-11 of the Response.

Response to Arguments. These arguments are not found to be persuasive. In reading of the as-filed disclosure, it is clear that the only transfection reagent that provides a transfection efficiency greater than that obtainable by electroporation, with regard to methods of transfecting human ES cells, is ExGen 500. Applicants have also acquiesced that the results in the as-filed disclosure provide this guidance. See p. 10, 1st full ¶ of the Response. Thus, it is determined that using a cationic, non-lipid polymer reagent, such as ExGen 500 is a critical or essential feature of the invention, *i.e.*, because this reagent is the only reagent that is shown to produce the claimed results, it is the only described reagent, within the group of claimed reagents that is described and supported by the as-filed disclosure. Furthermore, the Darr reference is a post-filing reference that is not germane to the instant rejection, this reference does not compare calcium phosphate transfection with electroporation in hES cells, and the specification does not contemplate this as a method to transfect the hES cells, within the context of the claimed invention. "The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed." MPEP §2163(B), emphasis added. It is incumbent upon Applicants to show that they had possession of the claimed invention, as filed, and as stated previously, because only ExGen 500 provides support for the claimed invention. The claims, as instantly amended, are not contemplated nor supported by the presently filed disclosure, because there is no support for transfection efficiency greater than that obtainable by electroporation, using the breadth of transfection reagents, as broadly claimed.

Written Description

Claims 1-3, 7-9, 11-13, 16, 17, 59 and newly added claims 60-63 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants' Arguments. Applicants argue that the advance of the presently claimed invention that defines over the prior art is that the discovery that while human cells cannot be transfected with reasonable efficiency using the same technique that is commonly and successfully used for transfecting mouse ES cells (*i.e.*, electroporation), human ES cells can be transfected with excellent efficiency using certain transfection reagents, such as ExGen 500. Applicants argue that they do not claim to have made any new advanced with respect to the exogenous nucleic acids, enhancers, promoters, or transcription activators that can be transfected into the ES cells using the present invention. Applicants argue that their experiments, which are directed to using fluorescent or antibiotic resistance proteins, establish a proof-of concept that some transfection reagents work more effectively than electroporation. Applicants argue that the art of transfecting cells with a polynucleotide, such that the gene expression before and after transfection is measurable different was well-within the skill of one of ordinary skill in the art at the time of the present invention. Applicants argue that their experiments merely show that this can be done at an efficiency greater than that obtainable by electroporation, but that the pluripotent character of cells are retained. Applicants argue that there is no reason to believe that any other polynucleotide might not be transfected in the same manner and achieve the same results as would be expected by one of ordinary skill in the art. Applicants argue that this concept is clearly considered to be part of the present invention, for example, with regard to the broad

definition of "expression altering sequence" (¶38 of the specification). See pages 12-14 of the Response.

Response to Arguments. These arguments have been fully considered, but are not persuasive. The claims require that the polynucleotide that is introduced into the hES cells be expressed, *as well as* retain the pluripotent character of the cells. Applicants have described using antibiotic resistance genes and marker genes, both of which are shown to retain the pluripotent characteristic of the resultant transfected cells. However, ¶38, which Applicants point to, clearly encompasses various genes, which may or may not retain the pluripotent character of the transfected hES cells. In particular, this paragraph recites genes that would be expressed in differentiated cells derived from human ES cells. Thus, this paragraph clearly encompasses genes that are expressed in differentiated cells, *i.e.*, cells that do not have a pluripotent characteristic. It is reiterated that the specification fails to describe any other species, within the genus of gene expression altering sequences that show measurably different gene expression after introduction of the polynucleotide, while retaining the pluripotent character of the cells, as instantly claimed and encompassed by the claims, with particularity, to indicate that Applicants had possession of the claimed invention. Accordingly, this rejection is maintained.

Enablement

Claims 1-9, 11-17, 59 and newly added claims 60-64 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for transfecting human ES cells, comprising introducing a polynucleotide that does not contain viral genes, into a population of human ES cells by transfection in the presence of a *cationic non-lipid polymer reagent*, wherein said polynucleotide is operably linked to a promoter that encodes a fluorescent protein, or an antibiotic resistance protein, and wherein the transfection efficiency is greater than that obtainable by electroporation;

the specification does not reasonably provide enablement for the breadth of the claims, which include transfection of hES cells in the presence of at least one transfection reagent selected from cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent, wherein the polynucleotide introduced into the hES cell is operably linked to a promoter and contains any gene expression altering sequence, such that the gene expression in the ES cells, prior to introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide, while retaining the pluripotent character to the cells. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Applicants' Arguments. Applicants' argue that one of ordinary skill in the art would expect that any kind of construct could be transfected into hES cells, using the present method, in view of the proof-of-concept that is present in the specification. Applicants argue that indeed, the laboratory of the present inventors has published many papers showing successful use of this process, with many different types of constructs. See pages 15-16 of the Response. Applicants provide Darr *et al.*, Lavon *et al.* (2006); Lavon *et al.* (2004); Urbach *et al.*; Dhara *et al.*, Schuldiner *et al.*, and Eiges *et al.* (2001). Applicants argue that these papers show that, despite the Examiner's comments, different constructs have been used, without undue experimentation, and that this confirms the fact that the proof-of-concept in the present specification would lead one of ordinary skill in the art to

understand that the full scope of polynucleotides, which are well known for the transfection of other cell types, can be used in the present invention. See pages 17-18 of the Response.

Response to Arguments. These arguments have been carefully considered, but are not found to be persuasive. The claimed invention requires, at minimum, two results. Firstly, that the transfection reagent provides a transfection efficiency that is greater than that which is obtainable by electroporation, and secondly, that the construct that is introduced contains a gene expression altering sequence, wherein the expression of the nucleotide is measurably different before and after transfection, while retaining the pluripotent character of the cells. It is with these two requirements of the claims that the Examiner addresses the prior rejection.

As stated in the prior Office action, the breadth of the claims encompass using any of the transfection reagents (cationic non-lipid, non-liposomal, or cationic lipid), however, the specification teaches that only ExGen, a cationic, non-lipid polymer reagent, provides a greater transfection efficiency than that obtainable by electroporation. The other reagents, Lipofectamine™, FuGene™, provided efficiencies within the range of electroporation, not a greater efficiency than that obtained by electroporation. Thus, the enabled scope of the invention, with regard to the particular transfection reagent that is used, in order to obtain the specific result required by the claim, is a cationic, non-lipid polymer.

Each of Applicants' provided references has been carefully considered, but not found to be persuasive.

Darr *et al.* teach using a calcium phosphate method to transfect wild-type ES cells with a human NANOG transgene. Darr *et al.* are not within the scope of the claimed invention, because they do not teach a transfection reagent that provides an efficiency greater than that of electroporation. Darr *et al.* do not teach comparison of their method with that of electroporation, thus, one of skill in the art could not predict if their transfection reagent (calcium phosphate) would provide the

transfection efficiency that is required by the claims. Furthermore, although Darr *et al.* show that NANOG transfected cells are capable of maintaining the hES cell pluripotent phenotype, this does not provide specific guidance to enable the large breadth of gene expressing altering sequences that are encompassed by the claims.

Lavon *et al.* (2006) is not within the scope of the claimed invention for the following reasons: Lavon analyzes the differentiation of hES cells to various pancreatic cell types. Thus, Lavon are not introducing a polynucleotide that has a measurably different gene expression after transfection and retains the pluripotent character of the hES cells. Furthermore, the claims require that the polynucleotide that is introduced into the hES cells does not contain viral genes (see last line of claim 1, for example). The construct that Lavon teach using is driven by the SV-40 *neo* selectable marker (see p. 1924, 2nd col., Plasmid Construction, last sentence). Thus, Lavon (2006) is not persuasive because it is not directed to the same scope as the instantly claimed invention.

Lavon *et al.* (2004) is not within the scope of the claimed invention for the following reasons: Lavon analyzes the differentiation of hES cells into hepatic cells. Thus, they are not introducing a polynucleotide that has a measurably different gene expression after transfection and retains the pluripotent character of the hES cells. Furthermore, the claims require that the polynucleotide that is introduced into the hES cells does not contain viral genes (see last line of claim 1, for example). The construct that is used by Lavon *et al.* is driven by a SV-40 promoter (see page 232, 1st col., 1st ¶, Plasmid Construction).

Urbach *et al.* is only within the scope of the claimed invention, in that they teach using a cationic non-lipid polymer (ExGen 500) to knock out a particular gene, the HPRT1 gene. Thus, Urbach *et al.* is only germane to the claimed invention in that they provide the appropriate transfection conditions, and they show that the protein that is expressed is an antibiotic resistance protein (hygromycin) and drug-resistant (6TG). Urbach *et al.* do not provide any further guidance with regard to

the breadth of the claimed invention, with respect to the various transfection reagents used, other than ExGen 500, or genes other than fluorescent proteins or antibiotic resistance genes.

Dhara *et al.* is not within the scope of the claimed invention because they use RET, a poly A-trap retrovirus vector. The claimed invention requires that the polynucleotide that is introduced into the hES cells does not contain viral genes. Furthermore, Dhara *et al.* provide no further guidance to utilizing any other transfection reagents, other than ExGen 500 (see p. 3996, 1st col., 1st full ¶).

Schuldiner *et al.* is not within the scope of the claimed invention, because they transfect hES cells with a vector that expresses the HSV-tk gene. The claimed invention requires that the polynucleotide that is introduced into the hES cells does not contain viral genes. Furthermore, the constructs that are described by Schuldiner either express the neomycin gene or the HSV-tk gene, and the eGFP gene (see p. 258, 1st col., Transfection and Establishment of Transgenic Cell Lines). Thus, Schuldiner only teaches the expression of viral genes, fluorescent proteins or antibiotic genes. Finally Schuldiner *et al.* provide no further guidance to utilizing any other transfection reagents, other than ExGen 500.

Eiges *et al.* are directed to the same experiments as those described in the instant specification. However, Eiges uses an SV40 promoter to drive their *neo* gene (see p. 518, 1st col., Plasmid Construction, last sentence). Thus, Eiges uses a construct that contains viral genes (which is not within the scope of the claimed invention), and further, does not teach the expression of genes other than what the Examiner has determined as the enabled scope of the claimed invention (a fluorescent protein or an antibiotic resistance protein).

It is maintained the prior rejection of record is proper with regard to the scope of the enabled invention. In particular, Applicants' disclosure, as well as the various Declarations provided by Applicants show that the state of the art of transfection is such that although one of skill in the art would be aware of the

various transfection reagents, as claimed, it was unexpected that using a particular reagent, ExGen™ (a cationic non-lipid polymer reagent) would produce a result of a transfection efficiency greater than all other reagents, including electroporation. See also, #6 of the Benvenisty Declaration filed 4/7/06. Furthermore, the working example in the specification only supports that ExGen is the only reagent that would produce a transfection efficiency that is greater than electroporation.

Furthermore, with regard to the particular gene expression altering sequence that would be introduced into the hES cells, the specification has only taught using fluorescent marker genes or antibiotic resistance genes, in order to practice the claimed method. Although the specification contemplates various sequences that could be used, the specification does not enable these sequences because there is no specific guidance or teaching with regard to particular enhancers or promoters, transcription activators that would both have a measurably different gene expression before and after transfection, and retain the pluripotent character of the hES cells.

Accordingly, in view of the unpredictable state of the art of transfection of hES cells, and Applicants' working examples, Declarations and arguments, it would have required undue experimentation, for one of skill in the art, to use any of the reagents, other than cationic non-lipid polymers, to transfect hES cells, with a gene expression sequence, whose expression is measurably different before and after transfection, while retaining the pluripotent character of the hES cells, and to obtain a transfection efficiency greater than that obtained by electroporation, as instantly claimed.

Claim Rejections - 35 USC § 112

The prior rejection of claims 1-9, 11-17 and 59 is withdrawn with regard to the term "with a transfection efficiency greater than that obtainable by means of electroporation". Applicants' arguments are found to be persuasive.

The prior rejections of claim 1 and 11, with regard to antecedent basis of the term "the nucleic acid" are withdrawn in view of Applicants' amendment to the claims.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 16 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 16 is unclear, because it recites "herpes simplex thymidine kinase" as a protein that can be encoded by the DNA. However, claim 11, from which claim 16 depends recites that the DNA sequence that is introduced does not contain viral genes. Herpes simplex thymidine kinase is obtained from herpes simplex virus, and thus, it is unclear how claim 16 limits claim 11, because it recites a viral gene. Appropriate correction is required.

Claim Rejections - 35 USC § 102

The prior rejection of claim 36 under 35 U.S.C. 102(a) or 35 U.S.C. 102(e) as being anticipated by Smith *et al.* is withdrawn in view of Applicants' cancellation of the claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject

matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Note: the Examiner re-applies this art because, although Applicants' have provided unexpected results with respect to utilizing a cationic, non-lipit polymer reagent (ExGen) to transfect hES cells, wherein the unexpected result is that the efficiency is greater than that of electroporation, this unexpected result is not found with respect to the other transfection reagents that are encompassed by the claims (non-liposomal, and cationic lipid agents). See also, MPEP §716.02 which state that, "Whether the unexpected results are the result of unexpectedly improved results or a property not taught by the prior art, the "objective evidence of nonobviousness must be commensurate in scope with the claims which the evidence is offered to support." Emphasis added. Thus, in the instant case, what is commensurate in scope with what is non-obvious is utilizing a cationic, non-lipid polymer reagent to transfect hES cells to result in a transfection efficiency greater than that of electroporation.

Claims 1-4, 6, 8, 9, 11-16, 36, 59-61are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith (U.S. Pat. No. 6,146,888, Reference AL of Applicants' Information Disclosure Statement, filed 3/26/03, cited previously) when taken with Ritter (Biochemica, No. 3, 1998, pages 47-49).

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. Smith teaches that another selectable marker that may be used is a product which is toxic, such as a suicide gene, for example, herpes simplex virus TK (see col. 2, lines 46-52). They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith teaches that a source of cells, including stem cells, is introduced with a selectable marker construct, wherein the selectable marker is adapted to operatively linked to an endogenous gene, or the introduction of a selectable marker construct, wherein the marker construct is linked to one or more gene fragments that provide differential expression. See Col. 3, lines 11-24. Smith teaches that promoter and cis-regulatory elements may be included in the expression construct. They further teach that a selectable marker may encode a cell surface antigen, or a gene product that allows for the purification of expression cells by panning of fluorescence-activated cell sorting (FACS). Smith teaches that a gene that displays a restricted stem cell expression pattern that can be used in their method is the Oct4 gene. They teach that Oct4 transcription is highly expressed in the expanding blastocyst and in the pluripotent cells of the egg cylinder. They teach that selectable marker genes, under the control of the Oct4 promoter may be applied to

the isolation of ES cell lineages. For example, the Oct4 gene promoter can be employed to drive stem cell specific transcription of a selectable marker, such as neomycin. See col. 5.

Smith do not specifically teach utilizing a non-liposomal reagent to transfect the stem cells. However, prior to the time of the claimed invention, Ritter *et al.* compare using lipofectin and Fugene to transfect 3T3 cells. See Materials, and Transient Transfections. They found that Fugene provided higher levels of transfection that lipofectin. See Figure 1.

Accordingly, in view of the combined teachings of Smith and Ritter, it would have been obvious for one of ordinary skill in the art to utilize the method of stem cell transfection, as taught by Smith, by utilizing either lipofectin or Fugene, as taught by Ritter, with a reasonable expectation of success. One of skill in the art would have been sufficiently motivated to make this modification, as Ritter teaches that DNA transfer has been substantially improved with the implementation of liposome-mediated technologies (see 1st ¶).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 1-4, 6, 9, 11-13, 15, 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* (cited above) when taken with Gibco BRL catalog (p. 350, 1992, cited previously)

Smith *et al.* are described above. They do not specifically teach utilizing a cationic lipid reagent for their transfection methods. However, prior to the time the claimed invention was made, the Gibco BRL catalog teaches LIPOFECTIN®, which is a liposomal formulation of a cationic lipid which is used to transfect a wide variety of cells, including human cells. See 1st ¶.

Accordingly, in view of the combined teachings of Smith and the Gibco BRL catalog, it would have been obvious for one of skill in the art to utilize the methods

of transfecting human ES cells, as taught by Smith, by using a transfection reagent, such as LIPOFECTIN®, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make such a modification, as it was an art-recognized goal to optimize transfection techniques of mammalian cells, and, as supported by the Gibco BRL catalog, that the LIPOFECTIN® reagent is a more efficient method of transfecting cells than calcium phosphate or DEAE-dextran transfection methods.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 5 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith when taken with Ritter as applied to claims 1-4, 6, 8, 9, 11-13, 15, 16, 36, 59-61 above, and further in view of Myers *et al.*

Smith and Ritter are described above. They do not specifically teach or suggest using a gene product that encodes a fluorescent protein such as green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

However, prior to the time the claimed invention was made, Myers teaches that bioluminescent and chemiluminescent reactions are used as analytical tools in various analytical applications, such as reporter gene studies. See p. 165, 2nd column, 1st ¶. Myers teaches that bioluminescent genes include the firefly luciferin and Renilla [see p. 165, 2nd column, lines 14-17 and #2]. Myers teaches that the gene for firefly luciferase has been cloned and is an effective reporter gene for studying transcriptional activity of cloned genomic sequences. See p. 168, #3.2.

Accordingly, in view of the combined teachings of Smith, Ritter and Myers, it would have been obvious for one of skill in the art to utilize the methods of

transfecting stem cells, as taught by Smith and Ritter, and transfect a construct encoding a fluorescent protein, such as Rennila protein, or luciferase, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was well-known in the art to use such fluorescent proteins as reporter genes and various other assays, and as supported by Myers, "Bioluminescent reactions are used as analytical tools in protein and nucleic acid blotting, in nucleic acid sequencing and hybridization assays, and in reporter gene studies ... The main advantages to these reactions are their simplicity and analytical sensitivity." See p. 165, 2nd column, 1st ¶.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable Smith when taken with Ritter as applied to claims 1-4, 6, 8, 9, 11-13, 15, 16, 36, 59-61, and further in view of Pascolo *et al.* (cited previously).

Simth and Ritter are described above, however, they do not specifically the knocking out of a genomic sequence in the ES cells, wherein the genomic sequence is selected from beta-2 microglobulin, HLA-1, HLA-2, or an INF receptor gene sequence.

However, prior to the time the claimed invention was made, Pascolo teach the generation of mice which are double knockouts of H-2D^b and mouse beta2 microglobulin and express human beta2 microglobulin and HLA-A2.1 monochains. See *Abstract* and p. 2043, col. 1-2, bridging ¶. In particular, Pascolo teach the various plasmids that were used to knock out the endogenous genes [see *Materials & Methods*] and the electroporation of the plasmids into mouse ES cells. See p. 2044, 1st column, *Cells & Transfectants*.

Accordingly, in view of the combined teachings, it would have been obvious for one of ordinary skill in the art to utilize the methods of transfecting human ES cells, as taught by Smith and Ritter, to knockout a genomic sequence, such as beta-2 microglobulin, as taught by Pascolo, with a reasonable expectation of success. One of ordinary skill would have been sufficiently motivated to make such a modification, as it was an art-recognized technique to knock-out endogenous genes to analyze gene expression and, and that in generating the double knockout H-2D^{b} /mouse beta2 microglobulin, Pascolo states, "This should facilitate the study of HLA class I-restricted responses compared to classical transgenic mice. One might home that the information gained with these animals will be of human relevance." See p. 2050, 2nd column, lines 4-7.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Thursday from 7:00 to 5:00 (Eastern Standard Time). Should the Examiner be unavailable, inquiries should be directed to Peter Paras, SPE of Art Unit 1632, at (571) 272-4517. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the Official Fax at (571) 273-8300. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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